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A multi-parameter *in-situ* water quality analyzer based on a portable document scanner and 3D printed self-sampling cells

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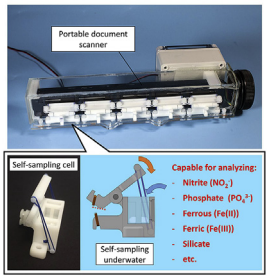
HIGHLIGHTS

- The self-sampling cells could automatically close and collect samples underwater.
- A portable document scanner was used as an *in-situ* multi-parameter detector.
- The glass wool was used as an adsorption-free reagent holder.
- The actuator-free design led to reduced complexity and a low unit cost.
- On-site *in-situ* experiments were carried out to prove the practicability.

GRAPHICAL ABSTRACT



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ABSTRACT

This research introduced a new low-cost and multi-parameter analyzer for *in-situ* measurements of typical nutrients in water bodies. The analyzer consisted of color detection and chromogenic reaction modules. The self-sampling action of the 3D printed sampling/reaction cells was achieved with the cooperative application of rubber bands and dissolvable thread. The target analytes in the collected water sample reacted with the chromogenic reagents that were diffused from the pre-placed glass wool in the cell, producing color compounds. A portable document scanner was employed as a multi-parameter *in-situ* detector to record the image of the colored solutions in all five cells simultaneously. Based on the image, the corrected grayscale values were derived for target analyte quantitation.

The relationships between grayscale values and concentrations of target analytes were established, and the temperature effects were studied. In addition, the practicability of the analyzer was demonstrated by *in-situ* experiments carried out in four different sites, including a creek, a river dock, a reservoir and a secondary settling tank in a wastewater treatment facility. The results indicated that the analyzer could be used for *in-situ* measuring of nutrients at μmol/L levels in the water. The nutrient concentrations obtained with the analyzer were comparable with those obtained with the standard methods. The presented analyzer provided new complementary ideas and methods for *in-situ* rapid measurement of nutrients and other target analytes in various water systems.

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1. Introduction

In water bodies, nutrient elements play important roles in

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aquatic ecosystems [1]. The main nutritive elements in aquatic environments include nitrogen, phosphorus, silicon, and iron. They exist in the forms of nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+) [2], phosphate ($H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-}) [3], silicate (SiO_3^{2-}, SiO_4^{4-} et al.) [4], ferrous (Fe(II)) and ferric (Fe(III)) [5,6], *etc.*, which are collectively known as nutrients. These nutrients are taken by aquatic biota to provide primary productivity. However, anthropic activities may lead to excessive nutrient discharge into aquatic systems (*e.g.*, lakes, rivers, or offshore coast), causing eutrophication and disturbing the ecological balance [7,8]. Therefore, monitoring nutrient concentrations is of great importance to environmental research and management.

Nutrient analytical techniques have been standardized and adopted by many countries for decades [9–14]. Most of these techniques are laboratory-based and adopt chromogenic reactions and spectrophotometric detections that are sensitive to specific analytes. To avoid target analyte degradation and contamination during sample collection, storage, and transportation, on-site or even *in-situ* analyses have been developed and become a hot research topic in recent years [15–18]. For rapid results, repeatability, and automatic sampling, the existing laboratory, on-site or *in-situ* analyzers mostly integrate flow injection analysis (FIA) techniques with classical chromogenic reactions [19].

The core components of a typical FIA-based analyzer [20] include several actuators for sampling and reagents injection, a reactor manifold, and a detector. Precise commercial actuators, such as multiport valves and pumps, are normally used for accurate and steady liquid transfer through a selected channel. The applied detector is usually a compact spectrophotometer with a flow-cell for determination of colored compounds produced in chromogenic reactions. The existing *in-situ* analyzers are usually only capable of detection of a single analyte at a time, since one detector normally can only focus on one analyte eluted from one channel. However, in a water body, multiple target analytes are related to and affect each other; thus, multi-parameter analyses are of great significance. Some commercial nutrient analyzers [21–23] are established to achieve multi-parameter measurement, in which independent detection modules are needed for different analytes even though they are packed in a single shell. In addition, these FIA-based analyzers are often complex and costly, due to the usage of multiple pumps and valves.

The combinations of document scanners or cameras and gels or films infiltrated with chromogenic reaction products have been proven applicable for determination of NO_2^-, NO_3^-, NH_4^+, PO_4^{3-}, Fe(II), chromium (Cr(VI)), and sulfide (S^{2-}) in aquatic environments or sediment pore water [24–31]. These methods are simple, fast, and cost-effective, as they do not require actuators such as pumps and valves. However, most of these reported studies are neither on-site nor *in-situ* analyzers, as further gel treatment and data acquirement are required in laboratory settings. In these reported studies, colors on gels or films are developed off-line with specific chromogenic reactions, and then scanned with common flatbed scanners for the image of a single target analyte.

In recent years, some water quality analyzers [32,33] have been developed in the authors' research group, based on the principles of simple design, lowered cost, and capability for *in-situ* multi-parameter measurements. In this research, a low-cost pumpless and valveless analyzer was presented for *in-situ* multi-parameter measurements of various nutrients. The self-sampling cells, which were enabled by the combination of rubber bands and dissolvable thread, could automatically close and collect samples after entering the water for *in-situ* reaction and detection. The glass wool, which acted as an adsorption-free reagent holder, was preplaced in the cells for providing chromogenic reagents. A portable document scanner was used as a multi-parameter detector to record the color changes of the samples *in-situ* simultaneously. The actuator-free design of the analyzer led to reduced complexity and a low unit cost. Proper experimental procedures were established, hence quantitation standard curves were plotted for each analyte. Finally, several on-site *in-situ* experiments were successfully carried out to prove the practicability of the analyzer.

2. Reagents and materials

2.1. Standard solutions and reagents

All chemicals used in this study were of analytical grade or better, and purchased from Alfa Aesar, U.S.A. unless stated otherwise. All solutions were made with freshly prepared deionized (DI) water (resistivity $\geq$ 18.2 MΩ cm) obtained from a Barnstead NANOpure Diamond UF water purification system (Thermo Fisher Scientific, U.S.A.). The preparations of all solutions and reagents were according to the US EPA methods [12,13], national standard methods of China [9–11], and other reported methods [34], with some modifications in concentrations.

(1) standard solutions of nutrients

Stock solutions of NO_2^- (100 mmol/L), PO_4^{3-} (10.0 mmol/L), silicate (counted as Si, 10.0 mmol/L), Fe(II) (0.01 mmol/L) and Fe(III) (20.0 mmol/L) were prepared and properly stored. All the working standards were freshly prepared from appropriate dilution of the stock solutions with DI water, except for Fe(III) with 0.01 mol/L hydrochloric acid (HCl) solution. More detailed steps of standard solutions preparation are provided in the Supporting Information S1.

(2) chromogenic reaction reagents and buffer solutions

Chromogenic reaction reagents for NO_2^-: A sulfanilamide (SAM, $C_6H_8N_2O_2S$) solution (10 g/L) was made by dissolving 5.0 g SAM in 500 mL 20% (v/v) HCl solution. A N-(1-naphthyl)-ethylenediamine dihydrochloride (NED, $C_{10}H_7NHCH_2CH_2NH_2 \cdot 2HCl$) solution (1.0 g/L) was obtained by dissolving 0.50 g NED in 500 mL DI water. A color developing reagent for NO_2^- (CR-N) was prepared by mixing SAM solution and NED solution at a volume ratio of 1:1.

Chromogenic reaction reagents for PO_4^{3-}: A molybdate (28 g/L)-antimonyl (0.60 g/L) mix solution was made by dissolving 14 g ammonium molybdatetetrahydrate ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$) in 500 mL 30% (v/v) sulfuric acid (H_2SO_4), and adding 0.30 g potassium antimonyl tartrate ($C_8H_4K_2O_{12}Sb_2$). A color developing reagent for PO_4^{3-} (CR-P) was prepared by diluting 37.5 mL molybdate-antimonyl mix solution to 250 mL with DI water. An ascorbic acid solution (AA, 25 g/L) for PO_4^{3-} reaction (AA-P) was made by dissolving 6.25 g ascorbic acid ($C_6H_8O_6$) in 250 mL DI water.

Chromogenic reaction reagents for silicate: A color developing reagent for silicate (CR-Si) (35 g/L) was prepared by adding 8.75 g ammonium molybdatetetrahydrate ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$) to 250 mL H_2SO_4 solution (0.40 mol/L). An ascorbic acid solution (AA, 60 g/L) for silicate reaction (AA-Si) was made by dissolving 15 g ascorbic acid ($C_6H_8O_6$) in 250 mL DI water.

Chromogenic reaction reagents for Fe(II): 0.4925 g ferrozine ($C_{20}H_{13}N_4NaO_6S_2$, ≥97.0%, Sigma-Aldrich, U.S.A.) was dissolved into 100 mL DI water to make a ferrozine stock solution (0.01 mol/L). A HAc-NH_4Ac buffer solution was prepared by slowly mixing 40 mL aqueous ammonia ($NH_3 \cdot H_2O$, 25% (w/w)) and 38.5 mL glacial acetic acid (CH_3COOH), diluting the solution to near 100 mL with DI water, adding a few drops HCl to adjust the solution pH to about 5, and then diluting to 100 mL. A color developing reagent for Fe(II) (CR-Fe(II)) containing 0.001 mol/L ferrozine was obtained by mixing

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10 mL ferrozine stock solution with 5.0 mL HAc-NH_4Ac buffer solution, and diluting to 100 mL with DI water.

Redox-chromogenic reaction reagents for Fe(II + III): The color developing reagent for Fe(II + III) (CR-Fe(II + III)) was the same as that for Fe(II) (CR-Fe(II)). The buffer solution was a mixed acidic solution (MA-Fe(II + III)) of ascorbic acid solution (AA, 50 g/L), glacial acetic acid solution (CH_3COOH, 1:60 (v:v)), and sodium hydroxide solution (NaOH, 12.5 g/L), at a volume ratio of 2:1:1. The solution pH was approximately 5.

All the above solutions were kept in high-density polyethylene (HDPE) bottles, wrapped up with aluminum foil to keep out light, and stored in a 4 °C refrigerator while not in use. All the prepared ascorbic acid solutions were used within three days.

2.2. Analyzer components and materials

The components, materials and consumables used to build the experimental analyzer included a portable handheld document scanner (I2, NTEUMM Electronic Tech, China), a microcontroller circuit board (Rysim, China) that contained a STM32F103C8T6 CPU (STMicroelectronics, U.S.A.), two rechargeable AA batteries (K-KJ17MCA4BA, Panasonic, Japan), a waterproof electrical junction box (Saip Electric Group, China), 0.3 and 3 mm clear polymethylmethacrylate (PMMA) plates (Xinnuode Tech, China), dissolvable sewing thread (Junhui, China), nylon screws and nuts (BRT, China), rubber bands (Xunoo, China), and glass wool (Jinqiao Analytical Instruments, China). The glass wool was soaked in 1 mol/L HCl solution overnight, then rinsed with DI water. After that, it was soaked in 1 mol/L NaOH solution for a few minutes, then thoroughly rinsed with DI water. Finally, it was dried at 100 °C in an oven and cooled to room temperature before use.

3. Design and construction of the *in-situ* analyzer

The analyzer consisted of a color detection module and a chromogenic reaction module, and the overall structure is shown in Fig. 1(a). The color detection module, which was the main body of the analyzer, included a scanner, a microcontroller circuit board, and their waterproof cases. When the scanner was triggered, it scanned through the transparent waterproof case and cell optical windows to produce strip-type images. A more detailed description of the analyzer's main body is available in Supporting Information S2. The chromogenic reaction module mainly consisted of five specially designed 3D printed self-sampling cells in series. Four of the five cells could be used for different chromogenic reactions, or for one reaction done in four parallels. The remaining cell was used as a reference for blank correction.

The details of the cells are illustrated in Fig. 1(b). The main

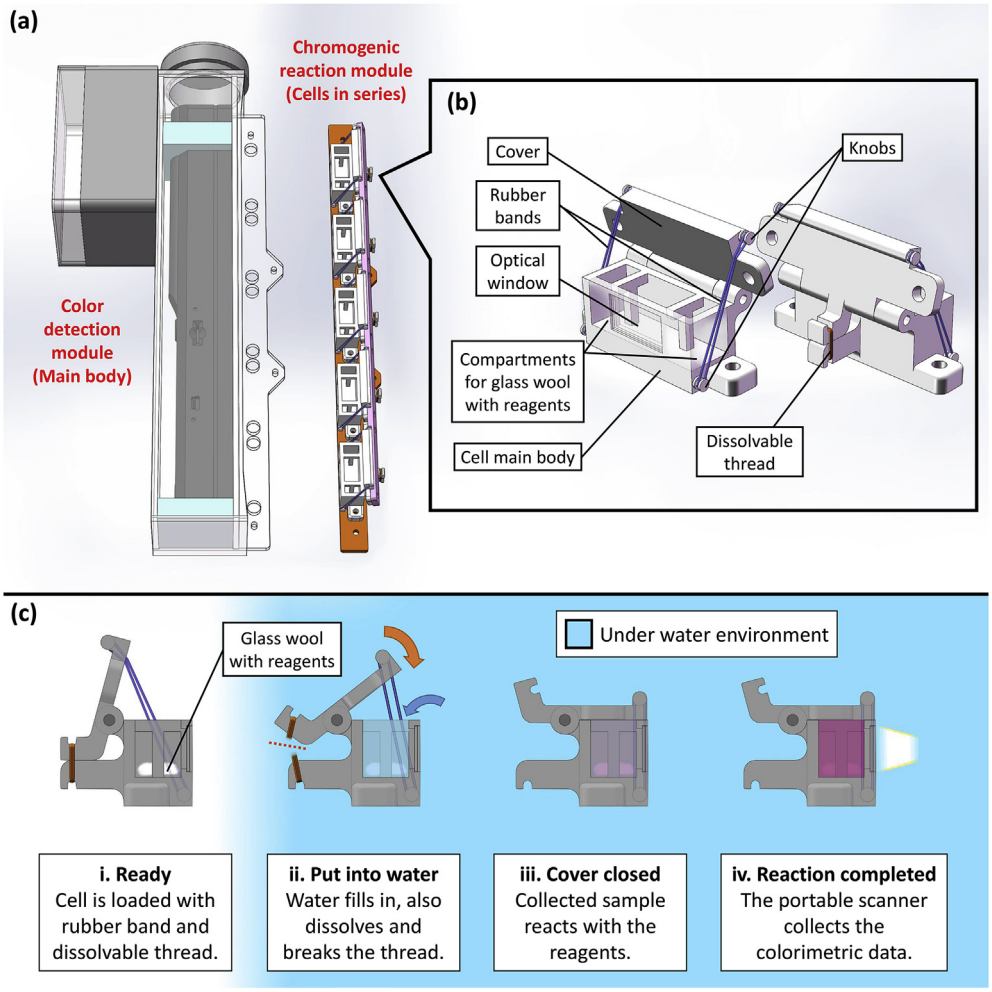

Fig. 1. The *in-situ* analyzer. (a) The overall structure of the analyzer. The analyzer consisted of a color detection module and a chromogenic reaction module. (b) Schematic of the 3D printed cell (effective volume = 2 mL, inner dimensions = 27 × 10 × 8 mm, optical window size = 14 × 7 mm). (c) Self-sampling procedures of the cell. The cell actions were enabled by the cooperative application of the dissolvable thread and rubber bands. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

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bodies of the cells were 3D printed with photopolymer resin via stereolithography apparatus (SLA) technique. Each cell, with an effective volume of 2 mL and inner dimensions of 27 × 10 × 8 mm, had three compartments. These compartments, with a volume ratio of 1:3:1, were separated by gratings that allowed the water sample and reagents to pass through them. The main compartment in the middle would be filled with the water sample for reaction, while the two smaller compartments on the left and right sides held glass wool with reagents. Glass wool was selected to hold the chromogenic reagents, as it does not affect or adsorb the target analytes and produced compounds. Furthermore, its characteristics of being low-cost and suitable for disposable use were also considered. The optical window of the cell, with a size of 14 × 7 mm, was a piece of 0.3 mm thin, transparent PMMA. When installed, the optical window of the cell faced the scanner. A pair of knobs on each side of the cell cover and the main body were used for rubber bands installations. A pair of hooks on the back of the cell was for the dissolvable thread. The applied dissolvable thread was made of poly(vinyl alcohol) (PVA), and could be rapidly dissolved in water at ambient temperature. Moreover, it was non-toxic, odorless, biodegradable and environmentally friendly.

The analyzer was able to work *in-situ* without external forces. The sample-collecting actions of the cells were enabled by the cooperative application of the dissolvable thread and rubber bands, hence the use of pumps, valves or other complicated actuators was not required. As shown in Fig. 1(c), once the prepared analyzer was deployed into water, the dissolvable thread rapidly broke and the cell covers were closed immediately under the tension of the rubber band to accomplish self-sampling. The design significantly reduced not only the complexity but also the cost, compared to other *in-situ* nutrient analyzers [21–23]. Then, the water sample reacted with chromogenic reagents diffused from the glass wool inside the cells to form colored compounds. Meanwhile, the portable document scanner, which serves as a multi-parameter detector on the five-in-series cells, captured the image for further analyte quantitation.

4. Operation procedures

4.1. Preparation

Approximately 15 mg glass wool was put into each small compartment on the left and right sides of all cells in the analyzer. Except for the reference cell, 100 μL prepared chromogenic reagent was added on the glass wool in each compartment of the four cells. The chromogenic reagents varied according to reactions for different analytes, as indicated in Table 1. The appropriate weight of glass wool and volume of chromogenic reagents were chosen based on the results of a preliminary experiment (see Supporting Information S3 for details). After adding the reagents, the cell covers were opened, and the hooks were tied with the dissolvable thread.

Table 1
Chromogenic reagents used for different target analytes. The abbreviations of the reagents were defined in Section 2.1. Exactly 100 μL of reagent was added on the glass wool in each compartment of the cells for reactions, except for the reference cell. The chromogenic reactions for NO_2^- and Fe(II) required only one kind of reagent in both compartments, while the rest required different reagents in different compartments.

Target analyte	Left side compartment	Right side compartment
NO_2^-	CR-N	CR-N
PO_4^{3-}	CR-P	AA-P
Silicate	CR-Si	AA-Si
Fe(II)	CR-Fe(II)	CR-Fe(II)
Fe(II + III)	CR-Fe(II + III)	MA-Fe(II + III)

Then, the cells were loaded with rubber bands on both sides. Finally, the prepared cell series (chromogenic reaction module) was mounted onto the main body of the analyzer (color detection module).

4.2. Measurement

The prepared analyzer was horizontally immersed into the water body for *in-situ* measurement. The analyzer was tightened onto a fixed location underwater to prevent floating or being flushed away, and shield with a dark cover to eliminate the possible effects of environmental lights.

As shown in Fig. 1(c), when touching water, the dissolvable thread broke in a few seconds. As a result, the cell covers were closed immediately under the tension of the rubber band to accomplish self-sampling. After that, the water sample inside the cell mixed with the chromogenic reagents diffused from the glass wool in the small compartments, and the reaction took place to produce color compounds.

The scanner was then switched on to collect images at a rate of 15 frame/min. Through the cell optical windows and transparent waterproof cases, the scanner collected an image of all five cells with each scan. After 20 min in the water, the scanner was switched off, and then the analyzer was retrieved to export data.

In the laboratory experiments of parameter optimization, to avoid using too much sample and also for convenience, the water samples were manually added into each cell rather than *in-situ* collected by the cells. The analyzer was covered with a dark box. The rest of the steps were the same as in *in-situ* measurements stated above, with the experiment duration also notably kept at 20 min.

In this research, dark covers had been used to shield the analyzer from the environmental lights for the best results. Some preliminary tests with the scanner showed that as long as the environmental light was not excessive (e.g. under laboratory light), its effect was much less than a measurement error and could be neglected. However, once the environmental light was very strong (e.g. under direct sunlight), a dark cover on the analyzer was still recommended to avoid affecting the measurements.

After each analysis, the inner walls of the cells were carefully cleaned with DI water and isopropyl alcohol to remove adsorbed colored compounds. In between each set of experiments, the cells were soaked in HCl solution (1 mol/L) overnight, and rinsed with DI water before the next use.

4.3. Data processing

There are no standardized data processing methods for colorimetric measurements based on reflective sensing. The reported methods adopt detectors such as flatbed scanners, cameras, and colorimetric sensors, and the chromogenic media can be papers, gels, films or solutions [26,28,35,36]. With reference to these works, an image processing method was established for the analyzer. In each of the red (R), green (G), and blue (B) channels, the portable scanner had different sensitivity to the color of the chromogenic products. Therefore, specific channels were selected for analyses of different colored products in this study. The G channel was used for pink compounds of NO_2^-, Fe(II), and Fe(II + III), while B channel for blue compounds of PO_4^{3-} and silicate, as these two channels were more sensitive to the respective colors.

The images were exported from the scanner for further analysis (see Supporting Information S4.1 for typical images of different analytes). Although the scanner could continuously collect images during the 20 min experiment period, only the last 10 images collected in the last 40 s of each measurement were selected for

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analyses, as per the procedures described in Supporting Information S4.2.

5. Performance of the *in-situ* analyzer

5.1. The establishment of quantitation standard curves

Typical nutrients in water were chosen as the target analytes in this study to demonstrate the practical use of the *in-situ* analyzer. In order to guarantee the reliability of the involved chromogenic reactions, all the reactions were adopted from US EPA methods [12,13], national standard methods of China [9–11], or other reported methods [34]. A target analyte reacts with its specific chromogenic reaction reagents to produce a colored compound, and the depth of the solution color reflects the concentration of the target. This study involved four kinds of reactions that were capable of analyzing five targets: NO_2^-, PO_4^{3-}, Fe(II), Fe(III) and silicate. The four reactions and their colored products are briefly described as follows.

The NO_2^- analysis was based on the Griess assay protocol where NO_2^- reacted with sulfanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride to produce a pink compound. The famous phosphomolybdenum blue (PMB) method for complexation of PO_4^{3-} led to a blue complex. The chromogenic reaction between ferrozine and Fe(II) was applied, and the complex was pink. For Fe(II + III) measurements, Fe(III) was reduced to Fe(II) before being determined. The silicate detection was based on silicomolybdenum blue compound. The developed colors could be quantitatively analyzed with both the developed methods and typical spectrophotometric methods [9–14].

A series of standard solutions with different analytes were prepared according to their common concentration ranges in aqueous environments. These samples were then analyzed with the same procedures described in Sections 4.2 and 4.3. The analyte reacted with the reagents for 20 min under the laboratory temperature conditions (20 ± 1 °C), and the grayscale values were taken. Since the reaction of silicate was both time and temperature dependent, silicate calibration curves with three different temperatures (10 ± 1 °C, 20 ± 1 °C and 30 ± 1 °C) were plotted. The analyzer with the sample and reagents was put in an incubator (Blue Pard, Hanghai YiHeng Scientific Instruments, China) to realize the designated temperatures. Afterward, the quantitation standard curves of corrected grayscale values versus concentrations were plotted for each analyte, as shown in Fig. 2. Note that ΔG was the corrected grayscale and used for quantitation, which is discussed in detail in Supporting Information S4.2.

In the literature where quantitation of the analyte is based on images, the standard curves are usually nonlinear or have very limited linearity within a certain narrow concentration range [26–29,36]. This is also indicated by the results gained from this study. As shown in Fig. 2, the standard curves of the five analytes are all quadratic. By definition, the determination limit refers to the limit that a specified quantity analysis can actually achieve. In this work, the concept of determination range was introduced to describe the detection ability of the proposed method. The concentrations corresponding to the lowest and highest ΔG values in the standard curve were the lower and higher limits of the determination range, respectively. From Fig. 2, it could be found that the determination ranges for NO_2^-, PO_4^{3-}, Fe(II), Fe(III) and silicate were 0.20–15.0, 1.00–15.0, 1.00–20.0, 1.00–15.0 and 25.0–125 μmol/L, respectively. Taking silicate as an example, the standard curve showed that when the concentration of silicate was higher than 125 μmol/L, the corrected grayscale did not increase as the concentration increased, indicating that silicate at a higher concentration would not be well quantified.

5.2. The effects of reaction temperature and reaction time and their correction

The chromogenic reactions between the target analytes and reagents could be affected by the reaction temperature and reaction time. In order to evaluate the uncertainties caused by the analyzer, the classic spectrophotometric methods for nutrient analyses were used in this section for reaction temperature investigation. A commercial spectrometer (DR 6000EDU, Hach, U.S.A. or V-1100D, Shanghai Mapada Instruments, China) was applied and the complexes of NO_2^-, PO_4^{3-}, Fe(II)/Fe(III), and silicate were detected at 540 nm, 880 nm, 562 nm, and 810 nm, respectively.

The reaction kinetic curves of five target analytes were studied at temperatures ranging from 10 °C to 30 °C, to cover most of the possible water temperatures in applications. The spectrophotometric cuvette also served as a reaction cell, and was placed in the water bath to keep it at the designated temperature for most of the time, except when it was being measured with the spectrophotometer. For each reaction, the absorbance was determined every 2 min since the mixing of the water sample with the chromogenic reagents started. The detailed kinetic plots, results, and descriptions are provided in the Supporting Information S5.1. The results are summarized below:

i. At all tested temperatures, the Griess reaction for NO_2^- (10.0 μmol/L) and the PMB reaction for PO_4^{3-} (10.0 μmol/L) reached equilibria within 14 min and 10 min, respectively (Figures S5(a) and (b)).
ii. The reaction times of Fe(II) (10.0 μmol/L) with ferrozine and Fe(III) (10.0 μmol/L) with reducing reagent and ferrozine were almost independent of the tested temperatures. Under any temperature between 10 °C and 30 °C, the reactions rapidly reached equilibria in less than 2 min, after ferrozine and reducing reagent were mixed with the water sample (Figures S5(c) and (d)).
iii. Except for silicate, all of the reactions were relatively fast and nearly reached equilibria in 14 min at all tested temperatures. The reaction temperature and time had impacts on the reaction rate of silicate (150 μmol/L) (Figure S5 (e)). It was noted that the silicomolybdenum blue reaction still could not reach complete equilibrium even after 1 h.

Considering the reaction time needed for the different target analytes, the duration of the measurement stage for the analyzer was set as 20 min. From the reaction kinetic curves of the five target analytes, it was found that the reactions of all the analytes except for silicate could reach equilibria before 20 min at all temperatures. Since the temperature of environmental water was not controllable during outdoor *in-situ* measurements, a temperature correction was necessary for silicate. In other words, the measured results had to be corrected based on a temperature calibration factor at a fixed reaction time. To determine the temperature correction factors, the relationship between the corrected grayscale ΔG and temperatures at 10 ± 1 °C, 20 ± 1 °C, and 30 ± 1 °C for silicate was established (Figure S6). After some calculations, as in Supporting Information S5.2, a temperature correction formula was derived.

5.3. Repeatability and accuracy of the measurements

The two analytes which easily reached reaction equilibrium, NO_2^- (10.0 μmol/L) and PO_4^{3-} (10.0 μmol/L), were selected as the representatives of the previously mentioned pink and blue compounds produced, respectively. Four reaction cells of the analyzer were filled with the produced compound solution and the other cell with DI water as a reference. Five measurements were

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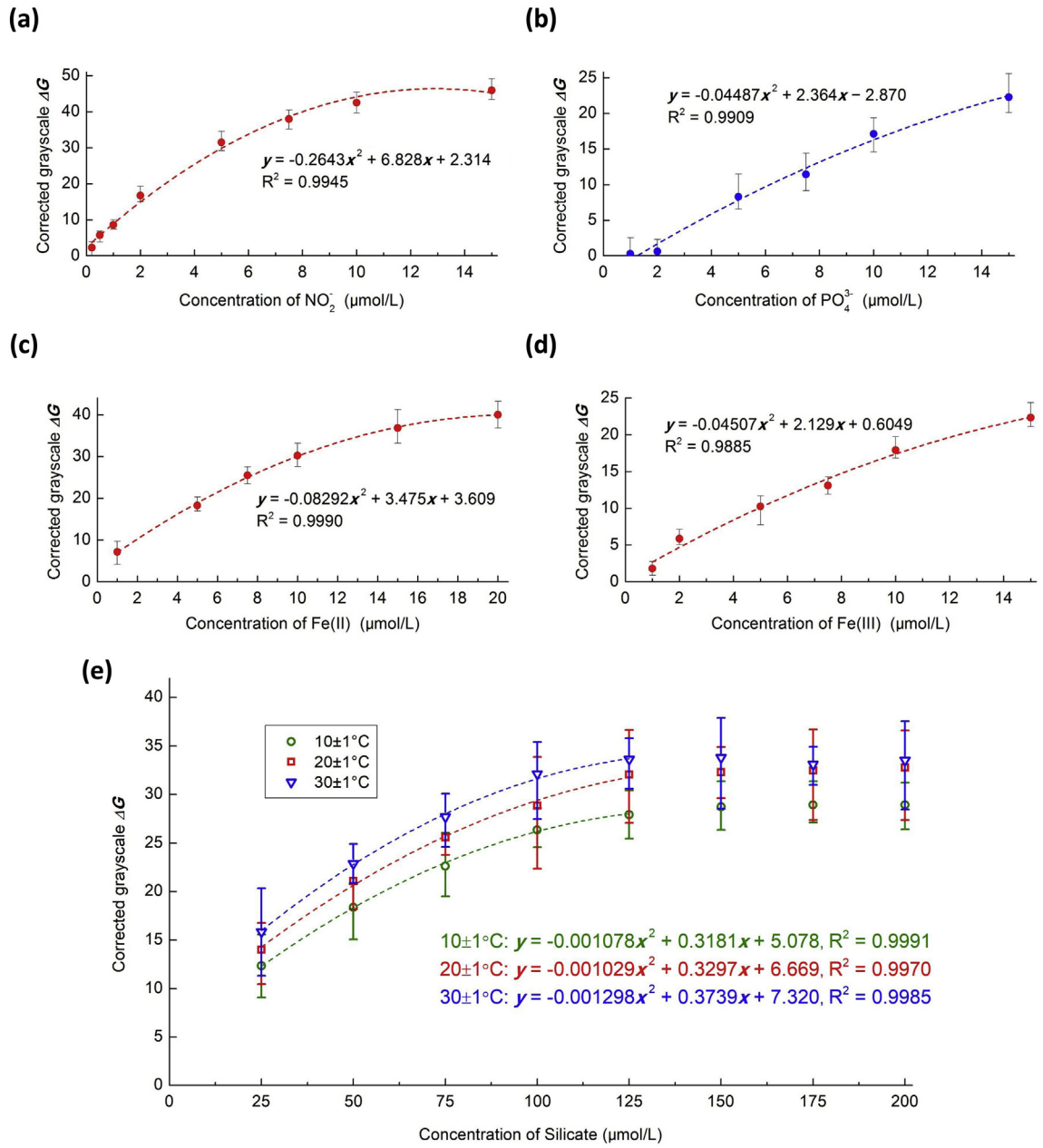

Fig. 2. The standard curves (n = 8): (a) NO_2^-; (b) PO_4^{3-}; (c) Fe(II); (d) Fe(III); (e) silicate. Note that ΔG was the corrected grayscale and used for quantitation.

conducted to examine the measurement repeatability of the analyzer. Each measurement gave four readings in a group, hence the inner-group relative standard deviations (RSD) was obtained. The average of the four readings from each measurement was taken, and the five average values were used to calculate the inter-group RSD. From Table 2, it can be found that the measurement RSDs within and among groups were less than 5%. The two analytes, NO_2^- and PO_4^{3-}, were measured as 9.38 ± 1.06 μmol/L and 10.35 ± 0.66 μmol/L, respectively, which were consistent with the true concentrations. The results of the measurements are listed in Table 2.

In addition, taking NO_2^- as an example to investigate the determination repeatability of the method, the analyzer was placed in a water tank filled with NO_2^- (5.00 μmol/L) solution. Five *in-situ* measurements were carried out, and NO_2^- was measured as 5.26 ± 0.58 μmol/L. The inner-group RSDs were 4.1%–5.4% and the

Table 2
Repeatability data of the measurements.

Measurement type	Target analyte	Inner-group RSD (%) (n = 4)	Inter-group RSD (%) (n = 5)
Produced compound solution	NO_2^-	2.5–3.2	2.9
	PO_4^{3-}	3.7–4.6	4.1
In-situ measurement in tank filled with sample (in laboratory)	NO_2^-	4.1–5.4	4.9

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inter-group RSD was 4.9%. Since the analyzer was applied to *in-situ* analyze the targets for rapid screening, an RSD of around or less than 10% was considered acceptable for semi-quantitation purposes.

6. *In-situ* application

Experimental applications were carried out *in-situ* to verify the practicability of the analyzer. Experimental site 1 was located at a creek in a community in northeastern Atlanta, U.S.A., and site 2 was at a river dock near the community. The date was March 23, 2019, and the weather was sunny. The outdoor temperature was between 15~20 °C, and the temperature of the water at both sites was 9.7 °C. Experimental sites 3 and 4 were located at Xiamen University, China. Site 3 was a reservoir and site 4 was a secondary settling tank in a wastewater treatment facility. The tests were done on October 31, 2019, a sunny day. The outdoor temperature was between 23~26 °C, and the temperature of the water at both sites was 21.0 °C.

The duration that the analyzer stayed underwater was 20 min, which was the time for the target analytes to react with the reagents. During the *in-situ* process, the analyzer operated smoothly. The sealing of the scanner compartment had no leakage. The captured images were clear. At the same time, water samples near the analyzer were collected with clean HDPE bottles, placed into a cooler (an insulated container full of ice), and brought back to the laboratory for further analysis within 8 h after sample collection. The water sample temperature in the laboratory was 20 ± 1 °C. The method for measuring water samples with the analyzer and the classical nutrient analytical methods were both used for analysis comparison under laboratory settings. The results of the *in-situ* measurement with temperature correction and the results of laboratory measurements are listed in Table 3.

From Table 3, some general conclusions could be made:

i. Silicon is one of the main diagenesis elements in the Earth crust at high content, resulting in high concentrations of silicate in all surface waters. In all four of the tested sites in both countries, silicate was detected. The silicate concentrations at the two sites in Xiamen University were higher than in Atlanta, and even beyond the quantitation range of the standard method. The determination of silicate with the analyzer was affected by the reaction temperature, thus temperature correction was needed (see Section 5.2 and Supporting Information S5.2).
ii. In the creek and river waters of Atlanta, where the running and shallow waters were more aerobic, NO_2^- could be easily oxidized to NO_3^- by the high levels of dissolved oxygen, and Fe(II) to Fe(III). Therefore, no NO_2^- and Fe(II) in the creek and river waters were detected with both the proposed analyzer and standard methods. On the other hand, reservoir water could be more anaerobic, and NO_2^- was determined to be present in the reservoir at Xiamen University.
iii. The concentration of PO_4^{3-} in most of the tested waters was very low, even lower than the limit of detection of the standard method and also the lower limit the analyzer's determination range. In the secondary settling tank where wastewater was treated and nutrients were concentrated, NO_2^- and PO_4^{3-} were well determined with both the analyzer and standard methods.
iv. Overall, the results obtained with the analyzer were comparable to those found with the standard methods, demonstrating that the analyzer was sufficient for semi-quantitative analysis and rapid in-field screening.

7. Conclusion and perspective

This research presented a novel *in-situ* analyzer for typical nutrients in aquatic environments, based on a portable scanner and chromogenic image analysis. Utilizing the scanner with a wide sensing area and five-in-series self-sampling cells, the analyzer was capable of rapid *in-situ* multi-parameter detection. Even more, the relationships between the grayscale values and concentrations of target analytes were established, and the temperature effects were studied. The performance tests of the analyzer indicated that the measurement RSDs of both inner-group and inter-group were about or less than 5%. The analytical results obtained with the analyzer during *in-situ* field applications also had relatively good comparability with those obtained with the standard methods in laboratory settings. These successful applications showed that the analyzer could be used in *in-situ* rapid screening of multiple nutrients in most waters, especially in farm waters, pond waters, and treated wastewaters, where nutrients are relatively high and semi-quantitation is allowed.

The notably appealing features of the analyzer were its easy operation without electricity or external forces to trigger and control the actuator, and its capability of continuously obtaining images with a simple scanner. With acceptable performance, the analyzer was significantly more cost-effective. For more potential

Table 3
Comparison of measurement results (μmol/L). Silicate was detected in all the four sites. Fe(II + III) was detected in the creek and river (Atlanta), while NO_2^- was detected in the reservoir (Xiamen University). In the secondary settling tank (Xiamen University), the nutrients were concentrated so that NO_2^- and PO_4^{3-} were well determined. Overall, the results obtained with the analyzer were comparable to those found with the standard methods.

Location	Measurement method	NO_2^-	PO_4^{3-}	Fe(II)	Fe(II + III)	silicate
Site 1 (creek, Atlanta)	Analyzer *in-situ*	*	*	*	2.87	40.6
	Analyzer in-laboratory	*	*	*	3.82	51.0
	Standard method	*	*	*	1.77	76.5
Site 2 (river, Atlanta)	Analyzer *in-situ*	*	*	*	*	65.6
	Analyzer in-laboratory	*	*	*	*	56.2
	Standard method	*	*	*	0.33	73.6
Site 3 (reservoir, Xiamen University)	Analyzer *in-situ*	0.30	*	*	*	124
	Analyzer in-laboratory	0.32	*	*	*	124
	Standard method	0.37	*	*	*	154
Site 4 (secondary settling tank, Xiamen University)	Analyzer *in-situ*	3.46	10.0	*	*	**
	Analyzer in-laboratory	3.50	9.20	*	*	**
	Standard method	3.67	9.09	*	*	***

Note: A "*" indicates below the lower limit of the analyzer determination range or the limits of detections of the standard methods (see section 5.1 for the ranges and limits). A "**" indicates beyond the higher limit of the analyzer determination range, and a "***" indicates that the absorbance measured with the standard method was higher than 2.5 and beyond the quantitation range.

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applications in further studies, the detection sensitivity, accuracy and precision could be greatly improved by optimizing the design of the analyzer and enhancing the imaging quality of the scanner. Even more, the optimization of data storage, transmission, and processing methods may also be helpful to enhance the overall performance of the analyzer.

In addition to being a novel analyzer and measurement method for nutrients in the water, the presented analyzer and obtained primary results provide new ideas and methods for *in-situ* monitoring of other chemical and biological phenomena in water systems. For example, it could be used to monitor pollution levels or screen pollutants, such as those resulting from the sudden leakage of colored wastewater or the explosive growth of algae, as long as some modifications and adjustment of the existing basis are made.

Author contributions

Beichen Lin conceived the study, designed and made the analyzer, performed most of the experiments, analyzed the data, and prepared the paper. Xing Xie and Miao Lu revised the paper. Jin Xu helped to modify the scanner, program the microcontroller, and do the *in-situ* analysis. Cecilia Yu helped to polish the English. Luodan Chen helped to do the laboratory analysis. All authors reviewed the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2019.12.034.

References

[1] J.F. Allen, W.B.M. de Paula, S. Puthiyaveetil, J. Nield, A structural phylogenetic map for chloroplast photosynthesis, Trends Plant Sci. 16 (2011) 645–655.
[2] T. Tyrrell, The relative influences of nitrogen and phosphorus on oceanic primary production, Nature 400 (1999) 525–531.
[3] C.R. Benitez-Nelson, The biogeochemical cycling of phosphorus in marine systems, Earth Sci. Rev. 51 (2000) 109–135.
[4] E. Struyf, A. Smis, S. Van Damme, P. Meire, D.J. Conley, The global biogeochemical silicon cycle, Silicon-Neth 1 (2009) 207–213.
[5] F.M.M. Morel, N.M. Price, The biogeochemical cycles of trace metals in the oceans, Science 300 (2003) 944–947.
[6] L. Norman, D.J.E. Cabanes, S. Blanco-Ameijeiras, S.A.M. Moisset, C.S. Hassler, Iron biogeochemistry in aquatic systems: from source to bioavailability, Chimia 68 (2014) 764–771.
[7] V.H. Smith, G.D. Tilman, J.C. Nekola, Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems, Environ. Pollut. 100 (1999) 179–196.
[8] V.H. Smith, Eutrophication of freshwater and coastal marine ecosystems - a global problem, Environ. Sci. Pollut. Res. 10 (2003) 126–139.
[9] Ministry of Environmental Protection of China, Water Quality-Determination of Nitrogen (Nitrite)-Spectrophotometric Method, 1987. GB 7493-87.
[10] Ministry of Environmental Protection of China, Water Quality-Determination of Total Phosphorus-Ammonium Molybdate Spectrophotometric Method, 1989. GB 11893-89.
[11] Ministry of Environmental Protection of China, The Specification for Marine Monitoring - Part 4: Seawater Analysis, 2007. GB 17378.4 -2007.
[12] US Environmental Protection Agency, Method 353.2, Revision 2.0: Determination of Nitrate-Nitrite Nitrogen by Automated Colorimetry, Environmental Monitoring Systems Laboratory, 1993.
[13] US Environmental Protection Agency, Method 365.1: Determination of Phosphorus by Semi-automated Colorimetry, 1993.
[14] J.D. Strickland, T.R. Parsons, A practical handbook of seawater analysis, in: second ed.Bulletin, 167, Fisheries Research Board of Canada, Ottawa, Canada, 1972.
[15] J.C. McIntosh, M.J. Hendry, C. Ballentine, R.S. Haszeldine, B. Mayer, G. Etiope, et al., A critical review of state-of-the-Art and emerging approaches to identify Fracking-Derived gases and associated contaminants in aquifers, Environ. Sci. Technol. 53 (2019) 1063–1077.
[16] M.Y. Lin, X.P. Hu, D.W. Pan, H.T. Han, Determination of iron in seawater: from the laboratory to in situ measurements, Talanta 188 (2018) 135–144.
[17] S.N. Zulkifli, H.A. Rahim, W.J. Lau, Detection of contaminants in water supply: a review on state-of-the-art monitoring technologies and their applications, Sens. Actuators B Chem. 255 (2018) 2657–2689.
[18] M.E.E. Alahi, S.C. Mukhopadhyay, Detection methods of nitrate in water: a review, Sens. Actuators A Phys. 280 (2018) 210–221.
[19] J. Ma, D.X. Yuan, K.N. Lin, S.X. Feng, T.J. Zhou, Q.L. Li, Applications of flow techniques in seawater analysis: a review, Trends Environ. Anal. 10 (2016) 1–10.
[20] J. Ŕuzicka, E. Hansen, Flow injection analyses: Part I. A new concept of fast continuous flow analysis, Anal. Chim. Acta 78 (1975) 145–157.
[21] EnviroTech Instruments, EcoLAB 2 Multi-channel analyzer system data sheet; http://www.labtech.com.mx/files/ecolab_2.pdf (accessed May. 2, 2019).
[22] SubChem Sensor Systems, SubChem analyzers; http://www.subchem.com/index.html (accessed May. 2, 2019).
[23] Systea S.p.A. WIZ (Water in-situ analyzer) Probe data sheet; http://www.systea.it/index.php?option=com_k2&view=item&id=223:systea-spa-wiz-probe&Itemid=175&lang=en (accessed May. 2, 2019).
[24] F. Cesbron, E. Metzger, P. Launeau, B. Deflandre, M.L. Delgard, A. Thibault de Chanvalon, et al., Simultaneous 2D imaging of dissolved iron and reactive phosphorus in sediment porewaters by thin-film and hyperspectral methods, Environ. Sci. Technol. 48 (2014) 2816–2826.
[25] P.R. Teasdale, S. Hayward, W. Davison, In situ, high-resolution measurement of dissolved sulfide using diffusive gradients in thin films with computer-imaging densitometry, Anal. Chem. 71 (1999) 2186–2191.
[26] C.R. Guo, M.J. Ma, D.X. Yuan, Y.M. Huang, K.N. Lin, S.C. Feng, In situ measurement of dissolved Fe(II) in sediment pore water with a novel sensor based on C18-ferrozine concentration and optical imaging detection, Anal. Methods 11 (2019) 133–141.
[27] Y. Yao, C. Wang, P.F. Wang, L.Z. Miao, J. Hou, T. Wang, et al., Zr oxide-based coloration technique for two-dimensional imaging of labile Cr(VI) using diffusive gradients in thin films, Sci. Total Environ. 566 (2016) 1632–1639.
[28] Q.Z. Zhu, R.C. Aller, Two-dimensional dissolved ferrous iron distributions in marine sediments as revealed by a novel planar optical sensor, Mar. Chem. 136 (2012) 14–23.
[29] A. Widerlund, W. Davison, Size and density distribution of sulfide-producing microniches in lake sediments, Environ. Sci. Technol. 41 (2007) 8044–8049.
[30] E. Metzger, A. Thibault de Chanvalon, F. Cesbron, A. Barbe, P. Launeau, D. Jezequel, et al., Simultaneous nitrite/nitrate imagery at millimeter scale through the water-sediment interface, Environ. Sci. Technol. 50 (2016) 8188–8195.
[31] E. Metzger, A. Barbe, F. Cesbron, A.T. de Chanvalon, T. Jauffrais, D. Jezequel, et al., Two-dimensional ammonium distribution in sediment pore waters using a new colorimetric diffusive equilibration in thin-film technique, Water Res. X (2019) 2.
[32] B.C. Lin, Z.Q. Xu, J.Q. Wang, M. Lu, A low-cost water quality monitoring prototype device with embedded chromogenic reagent capsules and dynamic colorimetric detection, Sens. Actuators B Chem. 252 (2017) 24–29.
[33] B.C. Lin, J. Xu, K.N. Lin, M.P. Li, M. Lu, Low-cost automatic sensor for in-situ colorimetric detection of phosphate and nitrite in agricultural water, ACS Sens. 3 (2018) 2541–2549.
[34] M.J. Verschoor, L.A. Molot, A comparison of three colorimetric methods of ferrous and total reactive iron measurement in freshwaters, Limnol. Oceanogr. Methods 11 (2013) 113–125.
[35] Y.B. Cho, S.H. Jeong, H. Chun, Y.S. Kim, Selective colorimetric detection of dissolved ammonia in water via modified Berthelot's reaction on porous paper, Sens. Actuators B Chem. 256 (2018) 167–175.
[36] X.Y. Bao, S. Liu, W.G. Song, H.W. Gao, Using a PC camera to determine the concentration of nitrite, ammonia nitrogen, sulfide, phosphate, and copper in water, Anal. Methods 10 (2018) 2096–2101.